# KOOTENAI FISH HATCHERY

## ANNUAL REPORT

**January 1, 1991 - December 31, 1991**

**Prepared by:**

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## INTRODUCTION

The Kootenai Tribal Experimental White Sturgeon Acipenser transmontanus Facility is located in Boundary County, Idaho, approximately three miles west of the small community of Bonners Ferry (Figure 1). The facility was constructed in the spring of 1991 as a resident fish substitution measure under the Northwest Power Planning Council's Columbia River Basin Fish and Wildlife Program [Section 900 (g)(1) 1987 (H); Action Plan, section 1403 (7.5)]. Funding for this facility was provided by Bonneville Power Administration under auspices of the Northwest Power Planning and Conservation Act (P.L. 96-501,1980). It will produce 1- and 2-year-old sturgeon for introduction into the Kootenai River. These juveniles are for experimental purposes to determine the lack of wild white sturgeon recruitment in the Kootenai River owing to the construction and operation of Libby Dam and federal facility operated by the U. S. Army Corp of Engineers. The facility will release juveniles and 2-year-old sturgeon in the spring to fall period. Larger fish will be fitted with sonic transmitters so they can be located and tracked after release into the river.

Staffing at the facility includes two permanent personnel, one 8-month temporary employee, and a 4-month bio-aide. There is no housing at the site, but there is a trailer pad ready for hook up.

## FACILITY CONSTRUCTION

The facility is a metal pole building 17.8 meters long and 12.5 meters wide. There are four circular tanks 3.06 meters in diameter and 1.53 meters high and ten circular tanks 1.53 meters in diameter and 1.22 meters high. An office, laboratory space, and a backup generator are also in the building.

## WATER SUPPLY

The facility has two water supply systems; Kootenai River water and Bonners Ferry city water. The Kootenai River water system is first pumped into a head-box at about 947 1/min and is used for adult holding, incubation, and rearing. The city water system is first de-chlorinated through two activated charcoal canisters. Then the water goes through a column packed with media [shot gun wads] and then into a head-box. This flow of 94.7 1/min is used for incubation, rearing, and recovery of female spawners. The water temperature varies by season in the Kootenai River system, getting as cold as 1°C in the winter and as warm 17°C in the summer. The city system also varies by the season getting as high as 17°C and as low as 4°C in the winter. A water heater is used in the winter which increases the water temperature to 17°C in three tanks, the flow capability of the heater.

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## BROODSTOCK COLLECTING

From May of 1991 through July of 1991, white sturgeon broodstock were collected from the Kootenai River between Fleming creek and Massacre Rock. Fish were captured on setline and by angling (Table 1). Captured fish were usually placed upside down in a stretcher suspended across the boat gunwales, with river water added as needed. Fish were then sexed in the field by making a 1-cm abdominal incision, viewing gonadal tissue with a veterinarian otoscope, or by inserting a flexible plastic tube and extracting developing oocyte by suction. Once the sex was determined and staged, it was decided whether to bring the fish to the facility or release it. A total of ten mature fish, two females and eight males, were collected and transported back to the facility. All fish were tagged with a 30-cm long spaghetti type floy tag pierced through the base of the interior of the dorsal fin. All fish were also pit tagged, weighed, and measured.

## STAGING BROODSTOCK

Adult female sturgeon brought to the facility were periodically examined for oocyte development and level of maturation. The fish to be examined were guided into a stretcher so the head of the fish was enclosed in the stretcher hood. Once the fish was secured on the stretcher, it was rolled ventral side up and inclined slightly towards the head, keeping the gills in water and the ventral surface dry. In preparation for examination, the abdominal area anterior to the vent was treated with 4% nitrofurazone antibacterial solution. Using a scalpel with a size 10 blade, a 1-cm incision was made along the ventral midline approximately three to four ventral scutes anterior from the vent. Care was taken to cut just through the interior cavity lining. An oocyte (egg) sample was removed by aspiration using 4-mm internal diameter tygon tubing. A sample of about 100 eggs was drawn into the tubing and placed into a 150-ml beaker containing 50 ml of chilled Leibovitz (L-15) solution medium (Appendix 1A). After the sample was collected, the incision was closed and sutured using a cruciate suture. Suture material used was Ethicon's cutting CP-2(888) reverse cutting edge swedged to a 70-cm sterile, chromic gut suture. Care was taken to leave some slack in the suture to allow for swelling of the tissue during healing. The incision area was then washed with a 4% solution of nitrofurazone. After the examination, the fish was released back in the 10-ft holding tank.

The four criteria we used to determine probability and time of optimal egg maturity of a potential spawner were: 1) appearance, shape, color, and atresia of eggs; 2) egg diameter through long axis; 3) position of the germinal vesicle (GV); 4) progesterone maturation assay producing GV breakdown (GVBD). As eggs develop, they generally change from light grey to dark grey or black and from round to oval or irregular shaped. The amount of atretic eggs in a sample indicates a female is reabsorbing eggs and may not continue to develop in a positive direction.

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Egg diameter through the long axis was measured by lining up ten eggs along a millimeter ruler. This measure can suggest that from staging to staging of an individual female, if egg diameter is increasing and, therefore, continuing to develop. Egg diameter varies with individual fish, but it is helpful in determining positive development.

A portion of the egg sample was placed in a 150-ml beaker with 50-ml L-15 medium, placed on a hot plate, and boiled for five to eight minutes. This solidified the yolk and fixed the position of the GV. The sample is then cooled and the GV position is determined by bisecting the eggs under a dissecting microscope. Each egg is held with a pair of Adson-Brown tissue forceps and cut along the animal-vegetal axis with a single-edged razor blade. The GV position and the yolk polarization could then be evaluated. Eggs were categorized from Stage 1 through 5. In Stage 1, the GV is located centrally, and Stage 5 is when the GV lies in the cortical ooplasm of the animal pole (Figure 2). Stages 2, 3, and 4 are a progression of the GV from central to the outer cortex of the animal pole. Eggs with a GV position of Stage 4 and 5 represent females with a good chance of being induced to spawn.

The most important criteria used in staging potential female broodstock was progesterone maturation assay of eggs. Eggs were removed from the chilled L-15 medium and placed into a 4-well tissue culture plate containing 20 ml of incubation medium each. Then 0.1 ml of predissolved progesterone maturation stock solution was added to two culture wells leaving two wells as a control. The tissue culture plate containing 25 eggs in each well was incubated for 24 hours at 15°C. The absence of a GV after exposure to progesterone maturation steroid for 24 hours is termed germinal vesicle breakdown (GVBD). After incubation, the sample was placed in separate 150-ml beakers, boiled for 5 to 8 minutes, and then cooled. A portion of each sample was fixed in 10% buffered formalin for future maturation criteria. The eggs were then bisected along the animal-vegetal axis and examined for GVBD. Normally, a female that will successfully spawn will exhibit GVBD in 80% or more of the eggs assayed.

## SPAWN AND SURVIVAL 1990

The first time a Kootenai River white sturgeon was spawned for culture purposes took place in July 1990. For more information on spawning and survival refer to: Apperson and Anders 1990.

## FIRST SPAWN 1991

Broodstock collection began in May, with broodstock being transported to the facility through July 11, 1991 (Table 1). All males captured were checked on the river by inserting a 5-cm tygon tube attached to the nipple of a 20-ml plastic syringe into the genital opening located just posterior to the anus. If

milt was collected (or their gonads were inspected using a veterinary otoscope, and determined that they were sexually mature), they were transported to the facility. One female, tag #1494, was captured on May 25 and transported to the facility where she was staged with an egg diameter of 2.8 mm and a GV position of Stage 4. A progesterone assay was performed on the eggs, with only 40% exhibiting GVBD, although the texture and color were both positive.

On June 7, she was staged again with more favorable results. Eggs had a diameter of 2.9 mm and the GV position was Stage 4-5 with 60% of the eggs exhibiting GVBD. She was staged again on June 17 with the following results: egg diameter 3.0 mm and >90% GVBD. Although egg diameter was rather small, it appeared she could be induced to spawn.

Hormone injections to induce spawning began on June 19. She was injected with a primary injection of 10% of a 0.1 mg/kg body weight dose of luteinizing hormone-releasing hormone analogue (LHRHa) at 2200 hours. Twelve hours later, she was given a resolving dose of 90% of the 0.1 mg/kg body weight LHRHa. Prior to the induction injections, she was placed by stretcher into a 1.0-m x .67-m x 3.0-m covered fiberglass holding tank. The holding tank allowed the fish to be injected under water, reducing stress and handling. This also makes for easier observation while waiting for ovulation.

Four males (#1189, #1499, #1495, and #1050) had milt when checked at 1500 hours on June 20. A total of 30 ml of milt was collected from each. A sample of milt from each male was checked by microscope for motility and time to death. The remaining milt was placed in ziplock plastic bags with pure oxygen and stored in a refrigerator on ice. Care was taken to keep the tubing, syringe, and surface area dry when collecting sperm.

Ovulation was expected between 24 to 48 hours post-resolving injection on June 21. Approximately 500 dark eggs were observed stuck to the bottom of the holding tank. The stored milt was again checked, but had been activated and was unusable. At 0200 hours, all the males were sampled and milt was collected from males #1495, #1499, and #1189. The female was put in the stretcher and placed on supports over the holding tank. A length of tygon tube connected to the hatchery water supply was placed into her mouth for aeration. The ventral side was disinfected with 4% nitrofurazone and a 10-cm incision was made along the midline to expose the egg mass. This process began at 0245 hours. The eggs were gently removed with a plastic spoon from the body cavity. A sample for contaminate analysis of at least 70 g was removed and frozen. The eggs were placed into five stainless steel bowls. This process took approximately 40 minutes. Three 10-g egg samples were collected and counted later to determine fecundity. She was thoroughly disinfected with 4% nitrofurazone, and the incision was closed with "Ethicon" PDSII violet monofilament (polydioxanone) suture swedged to a reverse cutting CP-1 curved surgical needle. A continuous suture of both the inside body cavity and outside body wall was used, which proved to be effective. She was placed back into the recovery tank at 0415 hours.

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Egg fertilization and processing began at 0330 hours. The coelomic fluid, which contained some blood, was removed from the eggs. Then the three 10-m1 sperm samples were poured into a bowl of 6,000 ml of water. This mixture was then stirred and added to the eggs and gently stirred with feathers until the eggs started to stick to the feathers, which took about 3 minutes. The bowls were drained off and the de-adhesive solution of Fullers Earth (Diatomacous Earth) mixed with water, which was set in flowing water for tempering, was then added to the eggs and again gently stirred until the eggs were no longer sticky. Constant monitoring of the egg temperature was done so when the egg mixture increased more than 5°C, it was poured off and new tempered mix was added. This process lasted 75 minutes. The eggs were thoroughly rinsed to remove excess material and placed into five MacDonald jars, four on city water with approximately 13,971 eggs per jar, and one on river water with approximately 12,653 eggs. The flow was set at 3.79 1/min/jar. This flow rate kept the eggs suspended but not rolling. A total of 2,600 ml of eggs were removed from the fish at 26.36 eggs/ml for a total of 68,536 eggs.

## SECOND SPAWN 1991

Female tag #01582 was captured on June 20, 1991 and was staged with an egg diameter of 2.8-2.9 mm and GV position at Stage 4-5, but approximately 15% of the eggs were atretic and irregular in shape. Approximately 60% of the eggs exhibited GVBD, but at this time, no males were available for spawning. On July 11, a flowing male was brought into the facility. The female was again staged with these results: oocytes diameter still at 2.8-2.9 mm and GV position at stage 4-5, but approximately 30% of the oocytes were atretic and irregular in texture and shape. Approximately 60% of the oocytes exhibited GVBD. The consensus at the steering committee meeting on July 11 was to go ahead and spawn the fish. The procedure was the same as the first spawn, with the exception of only 1 male to 1 female ratio. Also, the University of Idaho with the co-operation of Idaho Fish and Game Research crew collected sperm samples and tried cryopreservation experiments. The results of these experiments will be described in Apperson 1991. The female started ovulation at 0502 hours on July 14th, and spawning began at 0905 hours. At 0945 hours, she was transferred back into the recovery tank, with a total of 82,110 eggs collected. During spawning it was noted that a large number of eggs were atretic, white in color, and irregular in shape. Male #01597 had a low density of sperm, which also could have contributed to the nearly complete mortality of the eggs.

## INCUBATION AND EARLY FRY REARING

At 1100 hours, eight hours post-fertilization, egg samples were taken to check for early cleavage. Approximately 60% of the samples checked showed cleavage. Neurulation was checked at approximately 80 hours post-fertilization. The percent neurulation results were: 16.4%, 22%, 9%, 18.7%, for the 4

respective MacDonald jars using city water for incubation. The Kootenai River water incubation had 7% neurulation.

The eggs showed increased fungus growth on the dead eggs at 48 hours post-fertilization. The fungus infected eggs were more buoyant and easily removed by siphoning. The water flow was increased to roll the eggs and reduce fungus clumping. Hatching began nine days post-fertilization on Kootenai River water and ten days on city water. Hatching lasted for three days. The late-hatching larvae showed a high incidence of deformity. Total hatching produced 13,930 larvae on city water and 2,083 on river water (Figure 3).

Larvae were allowed to swim out of the MacDonald jars directly into the 1.22-m x 3.06-m emergence tank .61-m deep. The river water larvae swam out of the MacDonald jar directly into a 1.53-m diameter circular tank.

The larvae on the city water were then sampled and divided into 2,500 fish per tank lots. The tanks were 1.53-m diameter circular tanks .92-m deep. The feed initiation began on July 10, 1991 for the first spawn, or 8 days post-hatch. The larvae were not actively feeding at first, but the initiation to the active feeding seemed to help with getting the larvae on feed.

The larvae were started on Biokyowa larvae feed 250, 400, and 700. During the day, the fish were fed on the sides of the tanks by hand and double AA-100 brand fish sitters. At night they were fed only with automatic feeders (AA-100). When the larvae were approximately 0.5 gram per fish, 57 days old, they were progressively switched over to Rangen Soft Moist product in the 3/64 feed.

## FISH TRANSFERS

On July 12, a total of 3,525 larvae were transferred from the Kootenai Tribal Experimental White Sturgeon Facility to Sandpoint State Hatchery to gene pool the fish in case of problems with the new facility.

## MORTALITY AND SURVIVAL

The majority of the mortality occurred in the first 20- to 45-day period, primarily due to getting the larvae on artificial diets, changing the larvae to a less expensive diet, as well as handling mortalities during monthly inventories. The highest mortality (approximately 4,000-5,000 larvae), occurred when new personnel at the facility used improper techniques during feeding and cleaning operations.

Mortality comparisons between fish reared on city water, Kootenai river water, and Sandpoint State Hatchery are represented from hatch through December 31, 1991 (Figure 4). Mortality rates were uniform (nearly identical) for all three groups at 88.2% for city water, 89.0% for river water, and 87.6% at Sandpoint Hatchery.

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Growth comparison between fish reared on city water, Kootenai River water, and at Sandpoint State Hatchery are represented from hatch through December 31, 1991 (Table 2 , Figure 5).

The second spawn began to hatch 10 days post-fertilization. The procedure of egg handling was the same as previously discussed. Only 90 larvae hatched. These were transferred into a 1.53-m diameter circular tank, and all perished prior to active feeding.

## PUBLIC RELATIONS

Approximately 250 people attended the June 6, 1991 Open House of the facility. Guests included Bonneville Power Administration officials, Kootenai Tribal Council and Tribal members, Idaho Department of Fish and Game officials, and local residents. A luncheon was given and provided by the Kootenai River Inn.

Nearly 500 people toured the facility during 1991. Also, we have had requests from teachers in the nearby community of Bonners Ferry to bring their students out to tour, but we have been discouraging them until the spring of 1992 when we will have broodstock at the facility.

The facility was covered by most media outlets. It was featured on KREM and KHQ Television stations, as well as in an interview with KUID TV Moscow. Also, it appeared on "Outdoor Idaho," which was a feature program on public TV.

The Bonners Ferry Herald, The Kootenai Valley Times, and the Spokesman Review covered the open house. The two local newspapers ran several articles about sturgeon culture.

## RECOMMENDATIONS

1. Broodstock: Broodstock collected should be returned as soon as possible to the facility to reduce stress. Fish should be checked regularly to determine degree of maturation. At least two females should be at the facility one month prior to spawning. One of these females should have spawned at least once before.

2. Spawning: Have a male to female ratio of 3:1. Use three separate bowls to fertilize the eggs. Keep them sorted by the male's number from incubation through stocking. Transfer a portion of eggs to Sandpoint State Hatchery to hatch and rear on heated spring water.

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3. Rearing: When larvae have hatched out, get a beginning sample weight and then weigh out 2,000 fish per tank. Take a 10-fish sample by using MS222, get weight in grams and length in mm of the number of fish per tank. This should be done once a month for the first two months. Then inventory regularly on a once a month schedule.

## TIME ALLOCATION

The time the hatchery staff allocated to the different phases of hatchery management is represented in Figure 6.

## ACKNOWLEDGMENTS

We wish to thank everyone who helped with the spawning of the Kootenai white sturgeon at the Kootenai Tribal Experimental White Sturgeon Facility during the 1991 season.

Table 1. Kootenai River broodstock collection, 1991.

| Floy tag number | PIT tag number | Fork length cm | Weight kg | Date caught | Date released | Used in spawn | Sex | Method caught |
|---|---|---|---|---|---|---|---|---|
| 1495[a] | 7F7F425D2C | 120 | 13.75 | May 15, 91 | Jul 11, 91 | Yes | M | R & R |
| 1499[a] | 7F7F435E69 | 151 | 23.75 | May 22, 91 | Jul 11, 91 | Yes | M | R & R |
| 1189[a] | 7F7F120C23 | 173 | 32.75 | May 20, 91 | Jun 25, 91 | Yes | M | R & R |
| 1050 | 7F7F444410 | 155 | 31 | May 24, 91 | Jun 25, 91 | No | M | R & R |
| 1586 | 7F7F442840 | 140 | 25.5 | May 26, 91 | Jun 20, 91 | No | M | R & R |
| 1581 | 7F7FE64433E | 143 | 26.5 | Jun 6, 91 | Jun 25, 91 | No | M | R & R |
| 1584 | 7F7E645373 | 155 | 29 | Jun 28, 91 | Jul 10, 91 | No | M | R & R |
| 1494[a] | 7F7F453646 | 155 | 24.5 | May 25, 91 | Aug 12, 91 | Yes | F | R & R |
| 1582[b] | 7F7E46004B | 154 | 26 | Jun 20, 91 | Sep 18, 91 | Yes | F | Setline |
| 1597[b] | 7F7E5E0A37 | 155 | 31 | Jul 8, 91 | Jul 22, 91 | Yes | M | Setline |

[a]Used in first spawn.
[b]used in second spawn.

Table 2. Kootenai Tribe White Sturgeon Facility mortality and growth.

| Date | Morts | Percent survival | Weight k | Monthly gain k | gm/fish | Fish/lb | Length cm | **Avg temp** °C |
|---|---|---|---|---|---|---|---|---|
| City Water | | | | | | | | |
| Jul 8, 91[a] | 0 | 100.00% | 0.571 | | 0.041 | 11.073 | 1.34 | 14.5 |
| Aug 1, 91 | 708 | 90.98% | 0.4806 | -0.0904 | 0.052 | 8.731 | 1.45 | 16.7 |
| Sep 1, 91 | 7.469 | 17.83% | 1.621 | 1.1404 | 0.890 | 883 | 4.61 | 15.7 |
| Oct 1, 91 | 564 | 12.31% | 2.238 | 0.617 | 1.780 | 255 | 6.85 | 13.1 |
| Nov 1, 91 | 543 | 6.99% | 1.692 | 0.454 | 3.786 | 120 | 9.51 | 9 |
| Dec 1, 91 | 135 | 5.67% | 3.047 | 0.355 | 6.168 | 74 | 12.43 | 11.5 |
| River Water | | | | | | | | |
| Jul 8, 91 | 0 | 100.00% | 0.0687 | | 0.033 | 13.758 | 1.34 | 14.5 |
| Aug 1, 91 | 1.784 | 14.35% | 0.0184 | -0.0503 | 0.062 | 7.323 | 1.45 | 15.2 |
| Sep 1, 91 | 17 | 13.54% | 0.1331 | 0.1147 | 0.472 | 962 | 4.4 | 14.2 |
| Oct 1, 91 | 28 | 12.19% | 0.3226 | 0.1895 | 1.270 | 357 | 6.1 | 10.6 |
| Nov 1, 91 | 14 | 11.52% | 0.54 | 0.2174 | 2.250 | 202 | 7.43 | 7.7 |
| Dec 1, 91 | 11 | 10.99% | 1.1141 | 0.5741 | 4.865 | 93 | 9.7 | 4.7 |
| Sandpoint Hatchery | | | | | | | | |
| Jul 8, 91 | 0 | 100.00% | 0.0687 | | 0.033 | 13.758 | 1.34 | |
| Aug 1, 91 | 776 | 77.99% | 0.0184 | -0.0503 | 0.062 | 7.323 | 1.45 | |
| Sep 1, 91 | 1.862 | 25.16% | 0.1331 | 0.1147 | 0.472 | 962 | 4.4 | |
| Oct 1, 91 | 153 | 20.82% | 0.3226 | 0.1895 | 1.270 | 357 | 6.1 | |
| Nov 1, 91 | 27 | 20.06% | 0.54 | 0.2174 | 2.250 | 202 | 7.43 | |
| Dec 1, 91[b] | 7 | 20.50% | 1.1141 | 0.5741 | 4.865 | 93 | 9.7 | |

[a]Transferred 3,525 fish to Sandpoint Hatchery.
[b]Transferred 200 fish to Cabinet Gorge Hatchery.

KOOTTABS

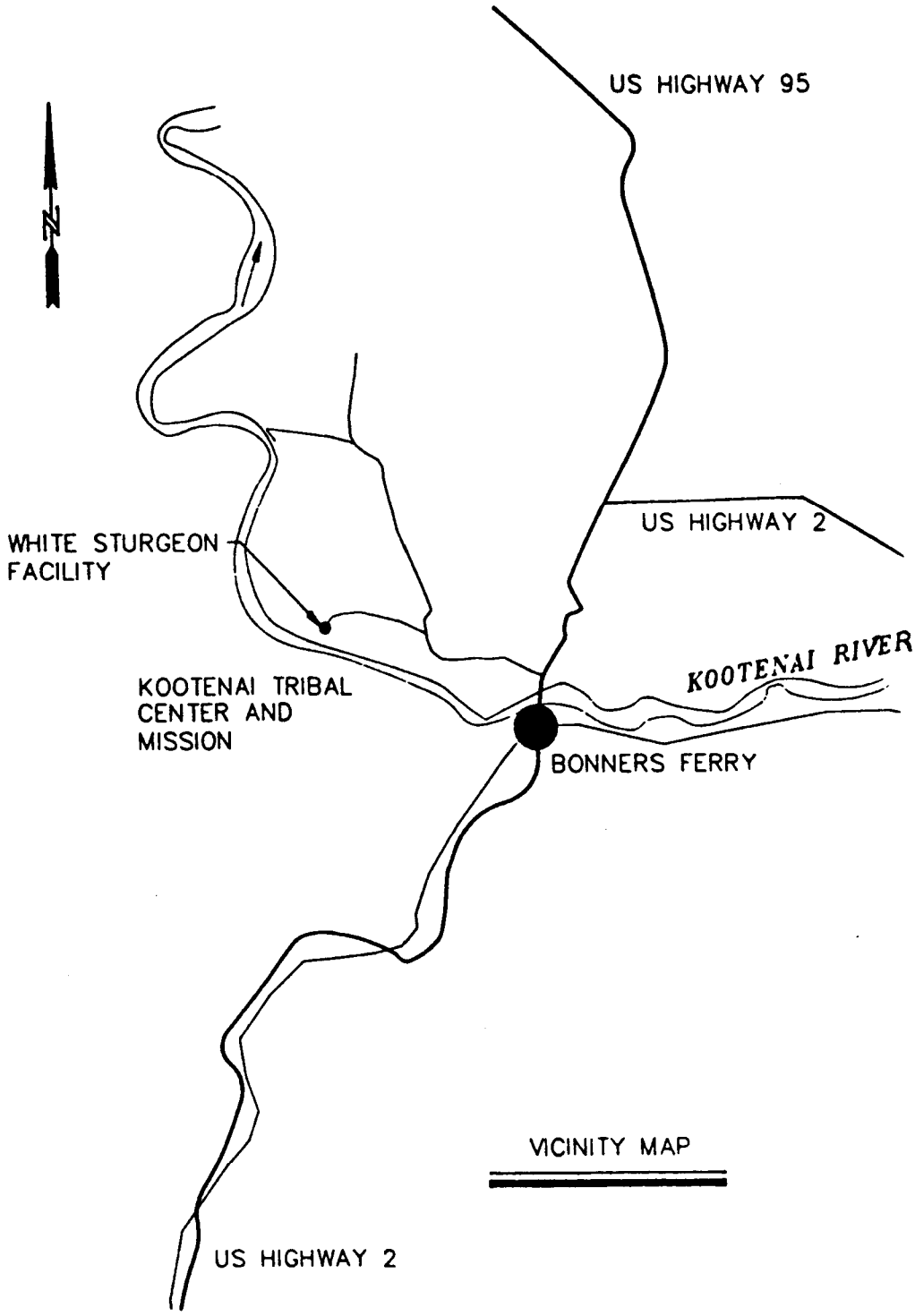


Figure 1: Location of Kootenai Tribal Experimental White Sturgeon Facility, RKM 241.3.

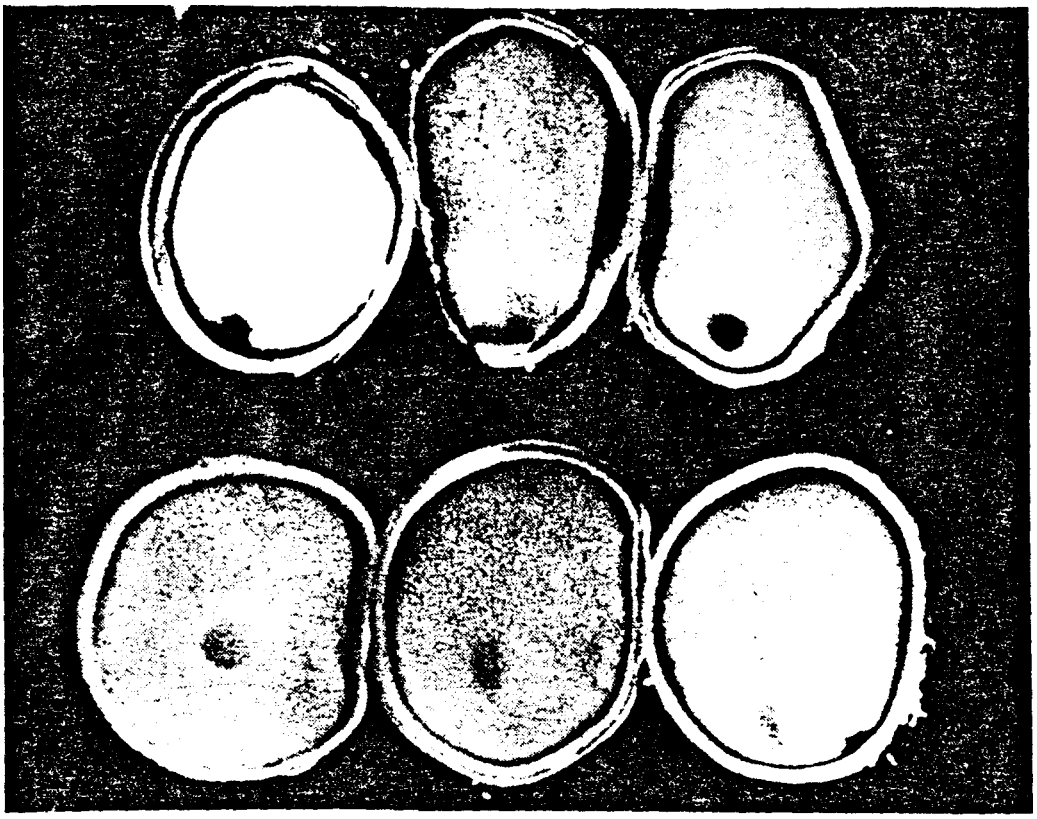

**FIGURE 2.**
Six bisected eggs showing their development and stage starting from the top left: 1st and 2nd eggs are in stage 5, the bottom row from left to right: 1st egg in stage 1, 2nd egg in stage 3, and the 3rd egg in stage 4. Picture from the Hatchery Manual for the White Sturgeon, U.of C. Davis.

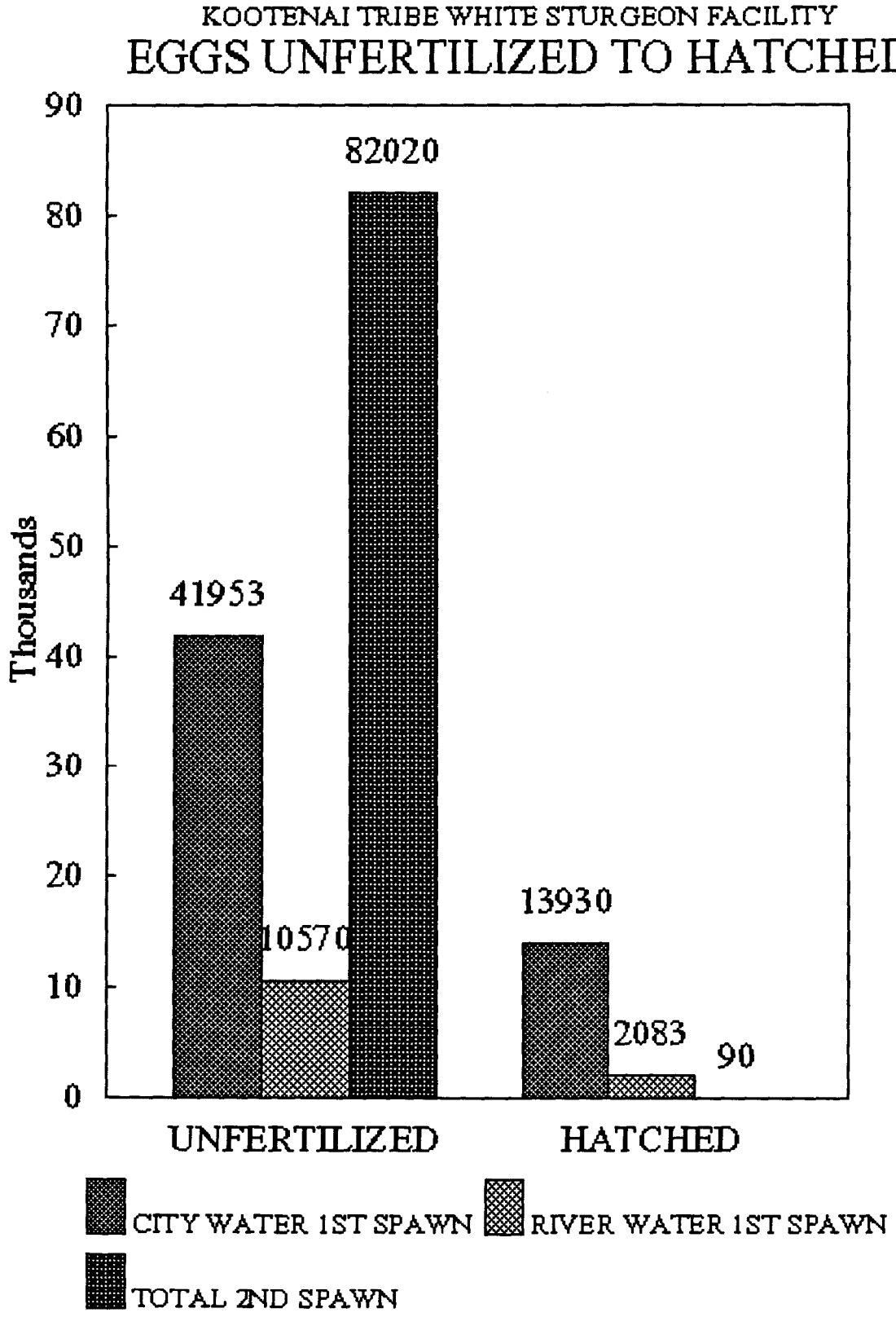
KOOTENAI TRIBE WHITE STURGEON FACILITY
EGGS UNFERTILIZED TO HATCHED
Thousands
90
80
70
60
50
40
30
20
10
0
41953
10570
82020
13930
2083
90
UNFERTILIZED
HATCHED
CITY WATER 1ST SPAWN
RIVER WATER 1ST SPAWN
TOTAL 2ND SPAWN



FIGURE 3

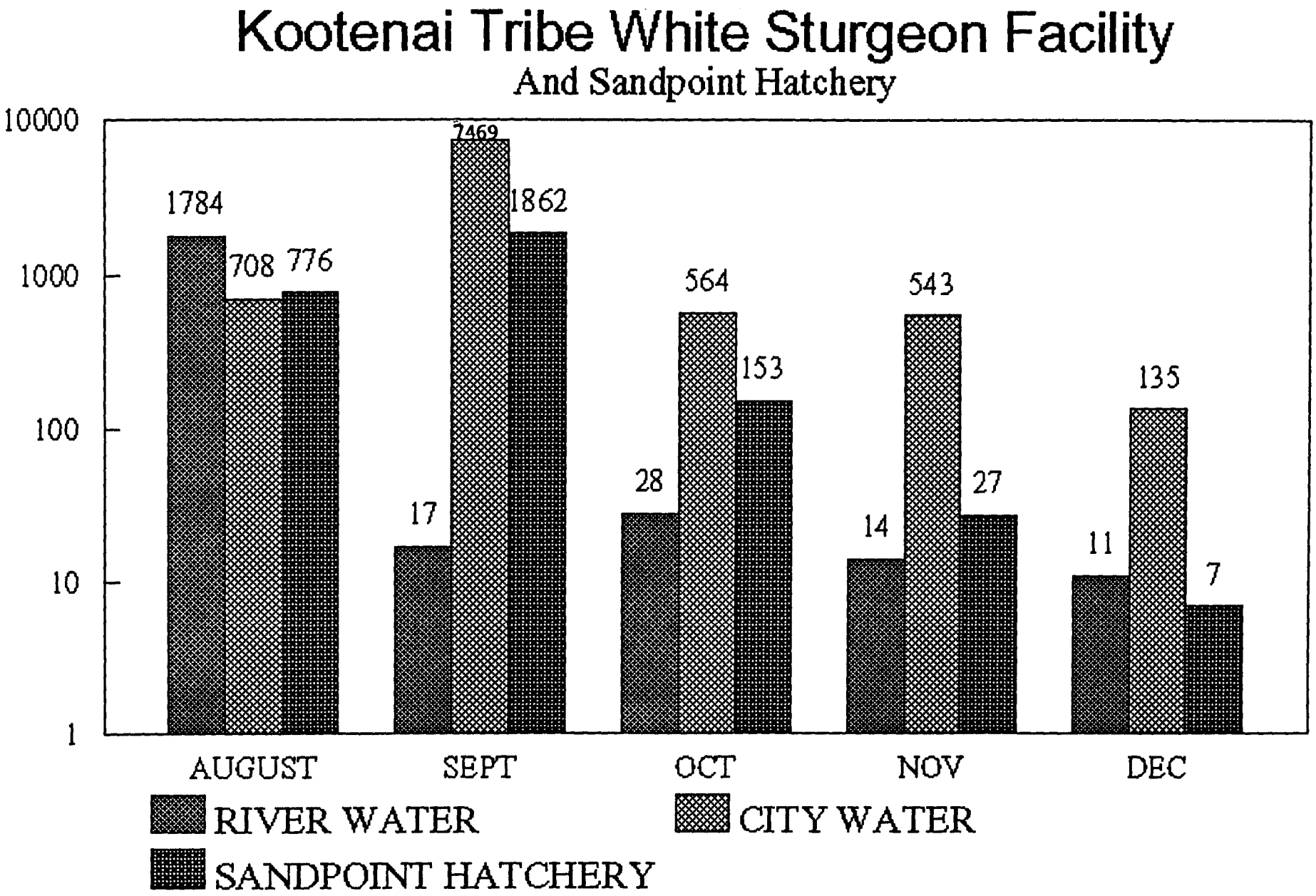


Figure 4: Mortality Comparison on different water sources

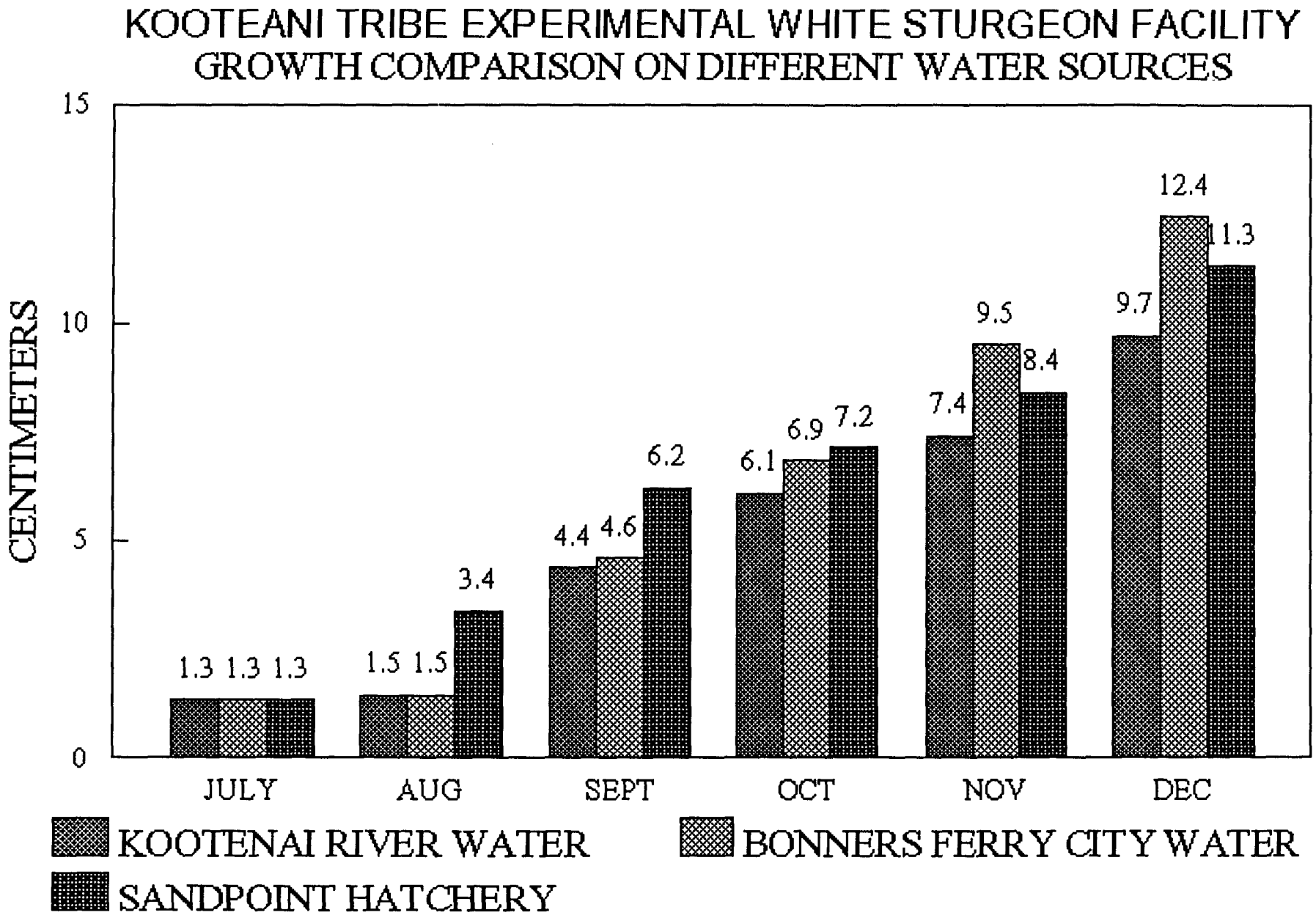


FIGURE 5: GROWTH COMPARISON ON DIFFERENT WATER SOURCES

# KOOTENAI TRIBE WHITE STURGEON FACILITY

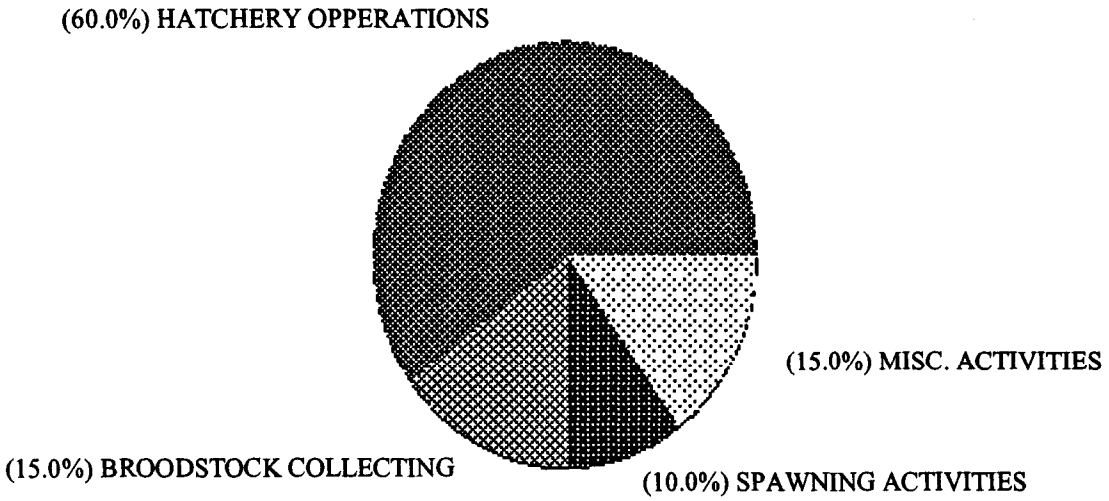




FIGURE 6: TIME ALLOCATION FOR FACILITY PERSONNEL 1991

## APPENDIX 1A

### Incubation Medium

If commercial products (such as L15 [Leibovitz] with LGlutamine, Sigma Chemical Company) are not available, a suitable medium can be prepared as follows:

| | | |
|---|---|---|
| NaCl | - | 6.5 g |
| $NaHCO_3$ | - | 2.0 mg |
| KCL | - | 250.0 mg |
| $CaC1_2$ | - | 300.0 mg |

Dissolve above chemicals in 1.0 L of distilled water. The addition of antibiotics is not required but is recommended. If antibiotics are used, they are added to the above volume as follows:

| | |
|---|---|
| Penicillin - | 500,000 units |
| Streptomycin - | 0.25 grams |

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